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The taxonomy and morphometry of squids in the family Loliginidae (Cephalopoda: Myopsida) from the Pacific coast of Mexico

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Abstract: The present research was a morphological and morphometric analysis of species from the family Loliginidae distributed in the Mexican Pacific and Gulf of California. These species are captured incidentally in shrimp fisheries and commercialized locally. Nevertheless, the number of species from the zone is uncertain, and there are numerous taxonomic problems in the group that limit the accurate identification of species and complicate the proper elaboration of the fisheries record. Morphological and morphometric analyses were performed using a canonical variable analysis (CVA) of 530 organisms. Six groups were separated a priori based on the shape of the funnel organ.

Based on the observed differences, four species were recognized: *Lolliguncula* (*Lolliguncula*) *panamensis* Berry, 1911, *Lolliguncula* (*Lolliguncula*) *argus* Brakoniecki and Roper, 1985, *Lolliguncula* (*Loliolopsis*) *diomedeae* (Hoyle, 1904), and *Doryteuthis* (*Amerigo*) *opalescens* (Berry, 1911). Two additional forms were recognized and named the following: *Lolliguncula* sp. 1, and *Lolliguncula* sp. 2. Three canonical variables (CV) explained 95.4% of variability among the groups in the analysis results. Variance percentages related to CV1, CV2, and CV3 were 61.9, 19.5, and 13.9%, respectively. A MANOVA analysis supported the statistically significant differences among the squid groups (Wilk's lambda = 0.023; $F_{(75.2447)} = 38.6$; $P < 0.0000$). In accordance with the analyses developed in this research, it is evident that there are morphological and morphometric characteristics that indicate a greater diversity of taxa are present compared to the list of species previously reported off the Pacific coasts of Mexico.

Key words: Mexican Pacific, discriminant analysis, cephalopod, funnel organ,

Squids from the family Loliginidae are represented by approximately 47 species grouped in ten genera and nine subgenera (Brakoniecki 1986, Nesis 1987, Vecchione *et al.* 2005, Jereb *et al.* 2010). Species in this family distribute from very shallow waters in bays and estuaries, over grass flats and coral reefs, to depths over 700 m, primarily in warm to temperate waters world-wide (Roper *et al.* 1984, Jereb *et al.* 2010). Loliginids are important in trophic webs of marine ecosystems as predators of small fishes and invertebrates and prey for demersal and pelagic fishes of commercial importance and marine mammals (Fields 1965, Recksiek and Frey 1978, Markaida and Hochberg 2005, Galván-Magaña *et al.* 2007).

A total of approximately 20 loliginid species are captured in coastal or near-shore artisanal fisheries, sometimes referred to as small-scale fisheries, and industrial fisheries worldwide. However, only four (*Doryteuthis* (*Amerigo*) *gahi* (d'Orbigny, 1835), *D.* (*Amerigo*) *pealeii* (Lesueur, 1821), *D.* (*Amerigo*) *opalescens* (Berry, 1911) and *Loligo reynaudii* d'Orbigny, 1841) are identified to species level in fisheries statistics (FAO, 1998). All the other species tend to be pooled in the genus *Loligo* Lamarck, 1798 in arrival notifications and global statistics (FAO 1998, Vecchione *et al.* 1998). The absence of detailed descriptions that guarantee accurate species identification is the principal cause of this problem, making management of these resources difficult. In Mexico, loliginids commonly caught by artisanal fisheries and as by-catch in shrimp trawl fisheries off the Mexican Pacific coast and in the Gulf of Mexico (Roper *et al.* 1984, Cardozo and Valdivieso 1988, Roper *et al.* 1995, Barrientos and García-Cubas 1997, Alejo-Plata *et al.* 2001, Alejo-Plata 2002, Sánchez 2003). Commercial fishing occurs during the entire year and demand continues to increase; therefore, defining the taxonomic status of the group through an exploration of alternative tools than those currently used is urgent. Thus, discrimination

between species will allow researchers to perform the biological studies necessary for the implementation of accurate management plans based on an individual approach of fisheries in Mexico.

The global taxonomic basis of the family Loliginidae remains complex and incomplete, particularly concerning specific and generic divisions (Vecchione *et al.* 2005, Cardoso and Hochberg, 2013). Therefore, a number of authorities reached consistency on the urgent necessity to conduct a comprehensive taxonomic review of this family (Voss 1977, Roper *et al.* 1983, Guerra 1992, Voss *et al.* 1998, Anderson 2000a, 2000b, Vecchione *et al.* 2005, Jereb *et al.* 2010).

Off the Pacific coast of Mexico and in the Gulf of California, five species have been reported: *Lolliguncula* (*Lolliguncula*) *panamensis* Berry, 1911; *Lolliguncula* (*Lolliguncula*) *argus* Brakoniecki and Roper, 1985; *Lolliguncula* (*Loliolopsis*) *diomedeae* (Hoyle, 1904); *Doryteuthis* (*Amerigo*) *opalescens* (Berry, 1911); and *Pickfordiateuthis vossi* Brakoniecki, 1996.

A few published studies of the group have been based on the spatio-temporal distribution of paralarvae (Okutani and McGowan 1969), records of stomach contents in fishes and marine mammals (Markaida and Hochberg 2005, Galván-Magaña *et al.* 2007), or general descriptions and distribution maps (Young 1972, Okutani 1980, Roper *et al.* 1984, Alejo-Plata 2002, Sánchez 2003). Because of the absence of taxonomic studies, there is considerable confusion on species identification and the total number of valid species in the region. In a study of invertebrates from the Gulf of California, *Loliolopsis chiroctes* Berry, 1929 and *L. diomedeae* are listed as separate species (Hendrickx and Brusca 2005), although *L. chiroctes* is a junior synonym of *L. diomedeae* (Voss 1971). Moreover, in 1980, Brakoniecki described *Lolliguncula tydeus* based on organisms from Central America and south Mexico. In a subsequent study, he considered *L. tydeus* as a junior synonym of *L. panamensis* (Brakoniecki 1986). Despite this correction, several authors have considered *L. tydeus* as a valid species (Cardozo and Valdivieso 1988, Alejo-Plata *et al.* 2001).

A taxonomic revision of the species in the family Loliginidae with distribution off the Mexican Pacific coasts and in the Gulf of California was performed in this survey based on the funnel organ, which presented noticeable visual differences among specimens. In addition, the shape of the mantle and fins, fin length proportions, tentacle lengths, arm lengths, tentacular club lengths, and sucker number in buccal membrane ribs, among other characters, were analyzed. In previous studies on squids, little taxonomic importance has been provided to the funnel organ for species discrimination, and it has only been referred to occasionally in the species description of a few families: Enoploteuthidae (McGowan and Okutani 1968, Roper 1964) and Loliginidae (Burgess 1967, Roper and Vecchione 2001). For some species from the tropical Eastern Pacific, the funnel organ shows an inverted "V" shape (Brakoniecki 1980, Barrientos-MacGregor 1985, Brakoniecki and Roper 1985, Cardozo and Valdivieso 1988, Barrientos and García-Cubas 1997, Alejo-Plata *et al.* 2001, Alejo-Plata 2002, Sánchez 2003). To confirm the funnel organ's effectiveness as a discriminating characteristic, a morphological and morphometric analysis was performed using multivariate analyses. These techniques have been employed with effective results on squid species discrimination using beaks (Martínez *et al.* 2002), statoliths (Neige 2006), and morphometric variables (Barón and Ré 2002).

MATERIALS AND METHODS

Field procedures

In total, 530 specimens were analyzed. Of this total, 404 were collected as by-catch in shrimp trawls off the Pacific coast of Mexico (Fig. 1) from the northern Gulf of California to the state of Oaxaca, including the western coast of the Baja California Peninsula (off Bahia Magdalena, Baja California Sur). The remaining 126 individuals analyzed were specimens housed in the cephalopod collection at the Santa Barbara Museum of Natural History (SBMNH), California, U.S.A. All specimens were preserved in 70% alcohol but had different treatments during fixation (Table 1).

Laboratory procedures

The specimens were identified and grouped according to the funnel organ. The funnel organ is an glandular structure fused to the internal surface of the funnel, generally a single W-shaped form in octopods and a dorsal inverted V-shaped component with opposed ventral oblong components in decapods (Roper *et al.* 1984) (Fig. 2C).

Morphological and morphometric analyses were performed to test the effectiveness of funnel organ discrimination. Sixteen measurements were obtained for each individual using a digital caliper to the nearest 0.01 mm (Roper and Voss 1983). The measurements included: mantle length (ML); mantle width (MW); middle mantle length (MML); right fin length (RFL); left fin length (LFL); fin width (FW); head length (HL); head width (HW); eye diameter (ED); arm I length (IAL); arm II length (IIAL); arm III length (IIIAL); tentacle length (TL); tentacle club length (TCL); funnel width (FuW); and funnel length (FL) (Figs. 2A, 2B). The lengths of both fins were included because a high percentage of *Lolliguncula* (*Lolliguncula*) *panamensis* specimens presented differences between left and right fin length.

The measurements were provided as proportions of mantle length (ML) using the mantle proportion length index proposed by Roper and Voss (1983). ML was considered because it is one of the few measurements that remain

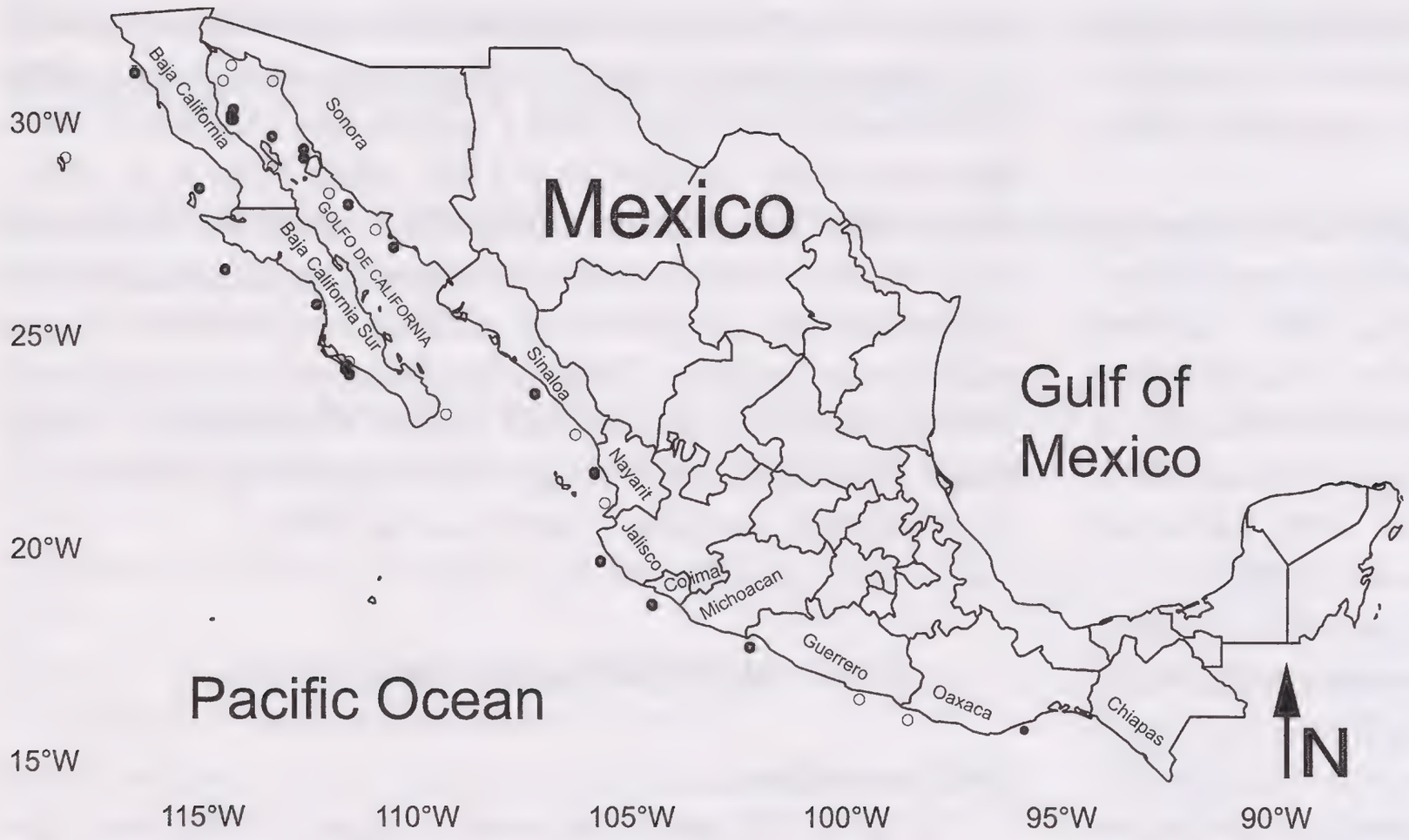

Figure 1. The location of sampling points off the Pacific coasts of Mexico and in the Gulf of California. Symbols: (●) Solid circles indicate present study; and (○) empty circles indicate specimens from the collection of the Santa Barbara Museum of Natural History (SBMNH).

consistent, independent from material condition, cause of death, or posterior treatment (Fields 1965). Additionally, an angular (x+0.01) transformation, was performed to all variables to stabilize the variance of the average during the proportion study, whereas it approached the variables in a normal distribution (Sokal and Rohlf 1985). This matrix was used for the multivariate analyses. A separate sex analysis was not shown because the sample size in five of the six analyzed taxa was relatively small. However, to avoid possible bias, hectocotylus measurements were not considered in this study. The hectocotylus (H) is a character with high taxonomic value in these species; nevertheless, given it is larger in size than the rest of the arms, its inclusion in the analysis would generate sex separation affecting the rest of the variables.

Statistical data analysis

A canonical variable analysis (CVA) was performed using 530 individuals and 15 variables (excluding mantle length). Statistical significance of the differences between groups was determined using Wilks' (λ) Lambda statistic, whose value when equal or close to 0 indicates a good discrimination model in contrast to 1, which represents a non-predictable model (Tabachnick and Fidell 1996). Moreover, the Mahalanobis (D^2) quadratic distances matrix was used for morphometric dissimilarity pattern analyses among the examined groups through an unweighted pair group method with the arithmetic mean analysis (UPGMA). The analyses were performed using the Statistica 8.0 program.

Voucher specimens

From the identified organisms, reference specimens were deposited in the collection of cephalopods housed at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) collection located in La Paz, Baja California Sur, Mexico.

RESULTS

Species accounts

***Lolliguncula (Lolliguncula) panamensis* Berry, 1911**

Synonyms: *Lolliguncula tydeus* Brakoniecki, 1980.

Funnel organ description: The dorsolateral patches were narrow and elongated with a crest on the superior section. The ventral patches were elongated and wide and thin to the inferior ends with a pronounced protuberance on the middle section. The superior section terminated in a triangular shape and was inferior with rounded tips. This section showed a fold on the internal section that covered 80% of its size. The funnel valve was wide with an identically sized superior fold (Fig. 3A).

***Lolliguncula* (*Lolliguncula*) *argus* Brakoniecki and Roper, 1985**

Synonyms: none

Funnel organ description: The dorsolateral patches were elongated and wider on the superior section. The ventral patches were elongated and narrow, the superior section was markedly pointed, and the inferior ends were rounded. The funnel organ showed a fold on the internal section that covered 40% of its size. The funnel valve was narrow with a pair of folds on its lateral sections that ended at the patches level (Fig. 3C).

***Lolliguncula (Loliolopsis) diomedeae* (Hoyle, 1904)**

Synonyms: *Loliolopsis chiroctes* Berry, 1929

Funnel organ description: The dorsolateral patches were elongated, wide on the superior section and thin on the inferior. The ventral patches were thin and elongated and slightly wider in the middle section. The superior section terminated in a triangular shape and the inferior ends were rounded. The funnel organ presented a fold on the internal section that covered approximately 70% of the patch size. The funnel valve was wide with an identically sized fold on the inferior section and another on the superior section that extended throughout the funnel width (Fig. 3B).

Table 1. Species, location, latitude and longitude, realized cruises list off the Pacific coast of Mexico and in the Gulf of California, treatment, and number of specimens. Abbreviations, cruises: UAENIP = Unidad Académica Escuela Nacional de Ingeniería Pesquera; CIBNOR = Centro de Investigaciones Biológicas del Noroeste; CUCSUR = Centro Universitario de la Costa Sur; SBMNH = Santa Barbara Museum of Natural History. Abbreviations, species: Do = *Doryteuthis* (*Amerigo*) *opalescens*; La = *Lolliguncula* (*Lolliguncula*) *argus*; Ld = *Lolliguncula* (*Loliolopsis*) *diomedeae*; Lp = *Lolliguncula* (*Lolliguncula*) *panamensis*; Lsp1 = *Lolliguncula* sp.1, and Lsp2 = *Lolliguncula* sp.2.

					Species					
Location	Latitude /Longitude	Cruise	Treatment	No. of specimens	Do	La	Ld	Lp	Lsp1	Lsp2
Sinaloa	23°65'N, 107°14'W	UAENIP-2009	96% Ethanol	44			44			
Nayarit	21°79'N, 105°75'W	UAENIP-2009	96% Ethanol	50			1	49		
Baja California (Gulf of California)	29°69'N, 113°33'W/ 30°09'N, 114°25'W	CIBNOR-2008	10% Formalin, then 70% Ethanol	107		2	22	83		
Sonora	27°09'N, 110°46'W/ 28°09'N, 111°53'W	CIBNOR-2008	10% Formalin, then 70% Ethanol	50			2	48		
Guerrero	17°68'N, 102°08'W	CIBNOR-2009	96% Ethanol	44			44			
Oaxaca	15°74'N, 95°63'W	CIBNOR-2011	96% Ethanol	26			26			
Baja California (Pacific coast)	31°19'N, 116°58'W/ 28°48'N, 115°06'W	CIBNOR-2011	96% Ethanol	3	2		1			
Baja California Sur (Pacific coast)	26°56'N, 114°43'W/ 25°75'N, 112°26'W	CIBNOR-2010	96% Ethanol	34	2	1	30	1		
Jalisco	19°70'N, 105°61'W	CUCSUR-2009	96% Ethanol	24			24			
Colima	18°69'N, 104°39'W	CUCSUR-2009	96% Ethanol	22			22			
SBMNH	various	various	10% Formalin, then 70% Ethanol	126	12	5	16	33	6	54
TOTALS				524	16	8	232	214	6	54

***Doryteuthis* (*Amerigo*) *opalescens* (Berry, 1911)**

Synonyms: *Loligo stearnsi* Hemphill, 1892

Funnel organ description: The dorsolateral patches were elongated, had a subtriangular shape, and rounded tips. The ventral patches were elongated and divided and wider in the middle section. The superior and inferior ends were rounded. It had a wide fold on the internal section that covered approximately 70% of the patch size without extending to the ends. The funnel valve was wide with a fold on the superior section that ended at the dorsal patch level (Fig. 3D).

***Lolliguncula* sp. 1**

Funnel organ description: The dorsolateral patches were wide and rounded on the superior section and pointed and curved at the inferior ends. The ventral patches were wide on the superior section and united in a "U" shape with thin inferior ends and were notoriously separated. It did not show folds in the middle section. The funnel valve was wide and thin without folds (Fig. 3E).

***Lolliguncula* sp. 2**

Funnel organ description: The dorsolateral patches were semi-circular and slightly wider in the superior section. The ventral patches were elongated and wide with an irregular shape, which was wider on the superior section with a small narrowing on the middle section that became wider again and finished into a tip. The superior section was narrow and pointed and showed a thin fold on the internal section that covered approximately 80% of its size. The funnel valve was narrow and not much folded (Fig. 3F).

Morphometry

We identified and grouped the 530 organisms into six groups (four species and two forms) based on funnel organ shape (Fig. 3). There were strong differences among the examined groups based on the results of the morphometric analyses. According to the canonical variable analysis, the initial three canonicals variables explained 95.4% of the variability among the groups. The variance percentages explained by CV1, CV2, and CV3 were 61.9, 19.5, and 13.9%, respectively (Fig. 4). The MANOVA analysis showed statistically significant differences among the squid groups (Wilk's lambda = 0.0239; $F_{(75,2447)} = 38.6$; $P < 0.0000$). The correct classification functions obtained for the six groups showed a matrix with a 77.0% correct assignment average, which, in general terms, indicates the high taxonomical integrity of the groups. Nevertheless, the *Lolliguncula* sp. 1 group presented a low (33.3%) correct assignment percentage, although this had only been confused with *Lolliguncula* (*Loliolopsis*) *diomedeae*. A greater sample size should better clarify these results. The highest assignation percentage was observed for *Lolliguncula* (*Lolliguncula*) *panamensis* with 97.1% (Table 2).

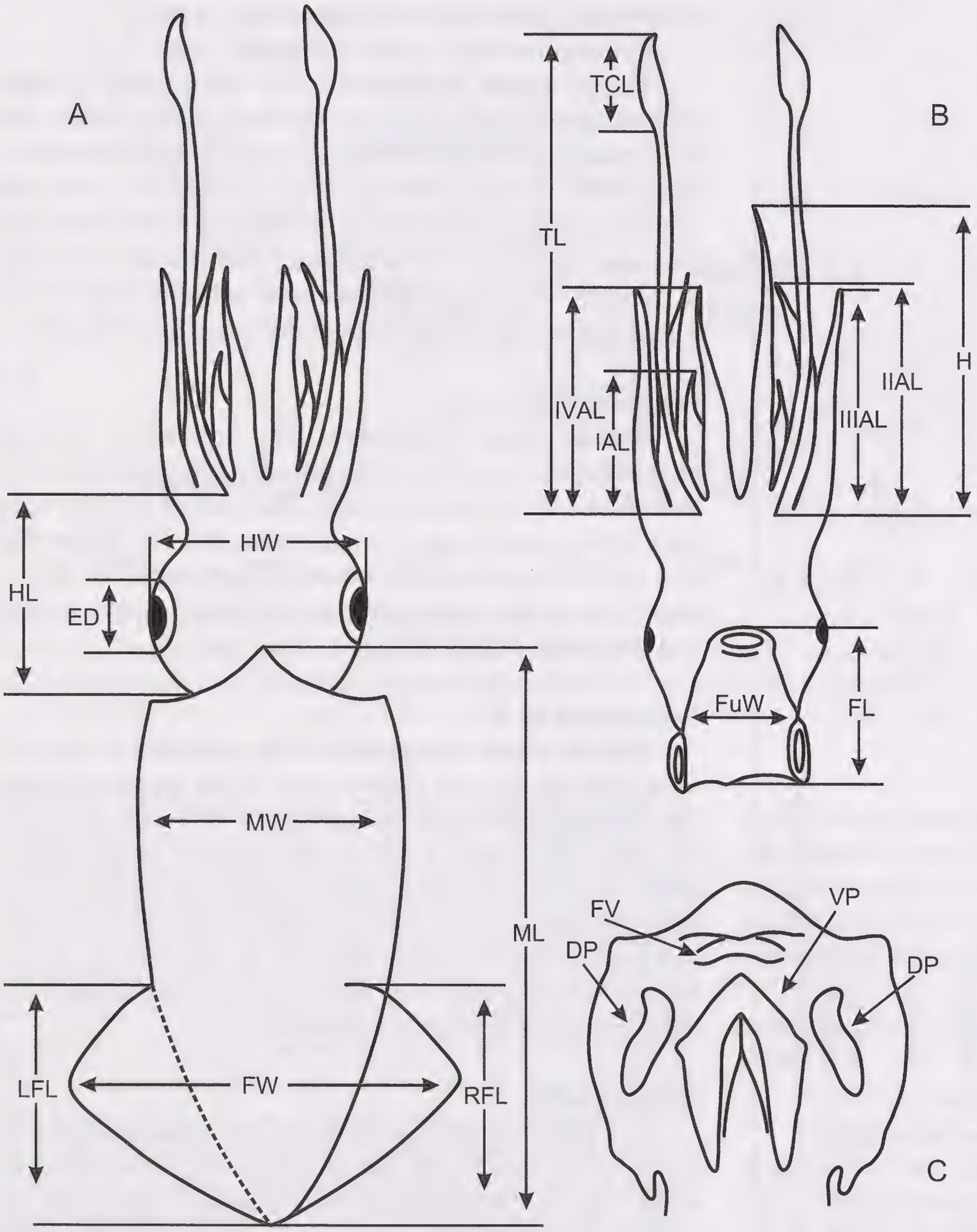

Figure 2. A diagram of the measurements. **A,** Dorsal view, **B,** ventral view, and **C,** structure of the funnel organ. Abbreviations: **DP,** dorsal pad, **ED,** eye diameter, **FL,** funnel length, **FuW,** funnel width, **FV,** valve funnel, **FW,** fin width, **H,** hectocotylus, **HL,** head length, **HW,** head width, **IAL,** arm I length, **IIAL,** arm II length, **IIIAL,** arm III length, **IVAL,** arm IV length, **LFL,** left fin length, **ML,** mantle length, **MML,** middle mantle length, **MW,** mantle width, **RFL,** right fin length, **TCL,** tentacular club length, **TL,** tentacle length (Roper and Voss 1983), and **VP,** ventral pad.

The obtained CVA value distribution showed a discrimination of two groups (explained by CV1): one group comprised *Lolliguncula panamensis* and the other by the rest of the species and the forms. The variables that showed high loading and CV1 included middle mantle length, right fin length, left fin length, tentacular club length, head width, and funnel width. Particularly, *L. panamensis* differed from the other species and the two forms by longer middle mantle and fin lengths. Given that unlike the rest of the species and the two forms, this species presents larger, wide and rounded fins. Although there was an overlap of shapes from CV2 and CV3, it was possible to observe agglomerations associated with each species. Thus, without considering *L. panamensis*, it is possible to distinguish *L. diomedeae* and the form *Lolliguncula* sp. 2 from the rest of the species and the form *Lolliguncula* sp. 1 in CV2. With respect to the observed loadings, the most important characteristics from the aforementioned differences were tentacle length, tentacular club length, head width, mantle width, arm lengths III, fin width, middle mantle length, right fin length, and left fin length. The results obtained from CV3 indicated that *L. argus, Doryteuthis opalescens*, and the form *Lolliguncula* sp. 1 were distinguished from other groups mainly because of the following characteristics: head width, fin width, head length, mantle width, tentacle length, and tentacular club length (Table 3).

The morphological similarity pattern obtained from Mahalanobis distances among the centroids of each group indicated that *Lolliguncula argus* was the most morphometrically divergent species whereas *Lolliguncula* sp. 1 and *Lolliguncula diomedeae* were among the most similar groups (Fig. 5). A great similarity between these two groups was also observed for the two classification functions, which misclassified one-half of the individuals of form *Lolliguncula* sp. 1 with *Lolliguncula diomedeae* (Table 2). However the distance topology does not represent an evolutionary history of this group, it is merely an assessment of the studied characters.

DISCUSSION

The loliginids are a diverse family of cephalopods (47 spp. and ten genera; Jereb *et al.* 2010), they represent a group with severe problems in systematics, which include the absence of taxonomic stabilization at higher levels (subgenera,

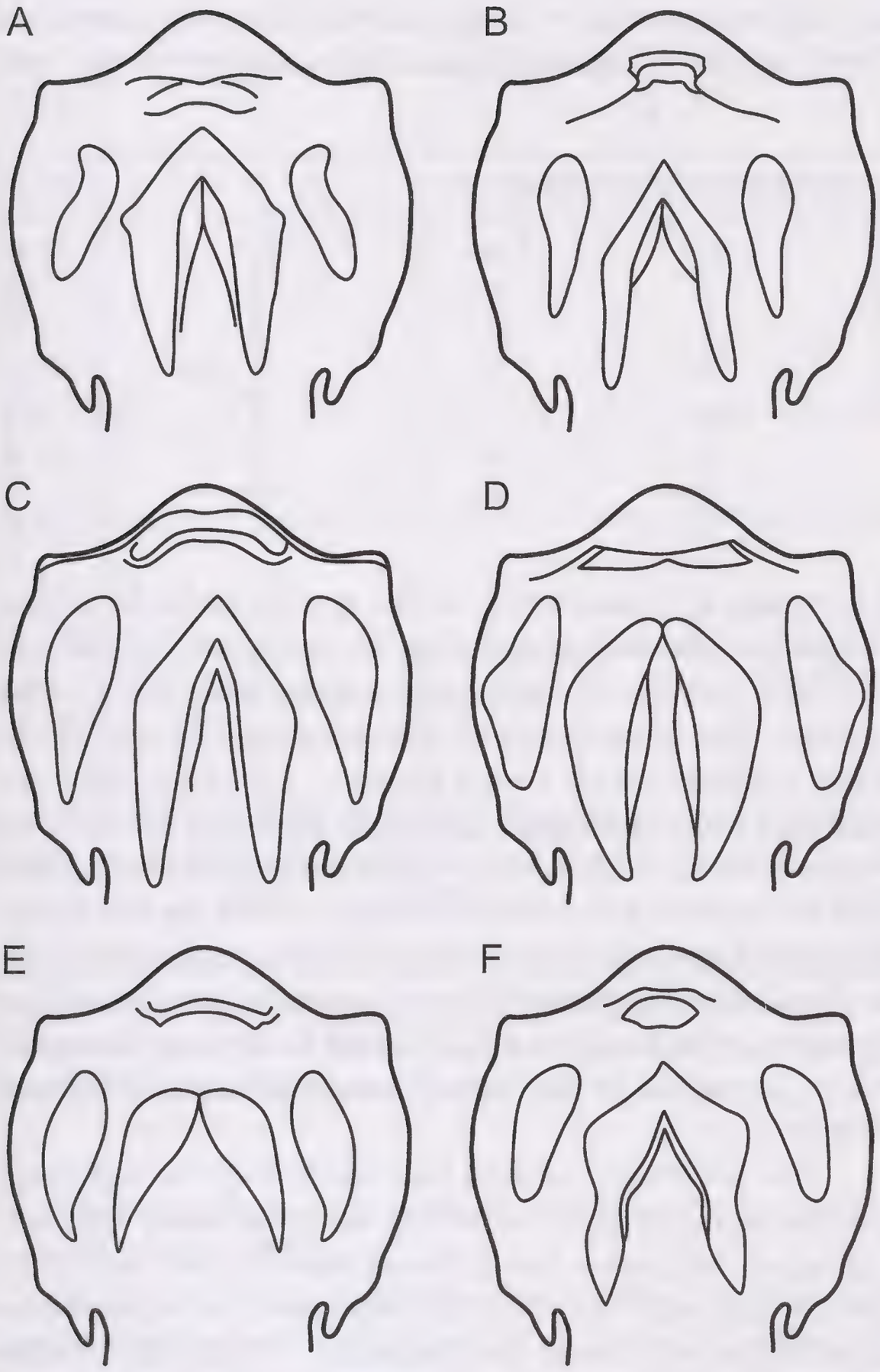

Figure 3. Diagram of the funnel organ differences: **A)** *Lolliguncula* (*Lolliguncula*) *panamensis*; **B)** *Lolliguncula* (*Loliolopsis*) *diomedeae* (SBMNH-60093 female ML 70.5 mm); **C)** *Lolliguncula* (*Lolliguncula*) *argus* (SBMNH-60043 female ML 61 mm); **D)** *Doryteuthis* (*Amerigo*) *opalescens*; **E)** *Lolliguncula* sp. 1 (SBMNH-64383 female ML 80.7 mm); and **F)** *Lolliguncula* sp. 2 (SBMNH-60083 female ML 44.3 mm).

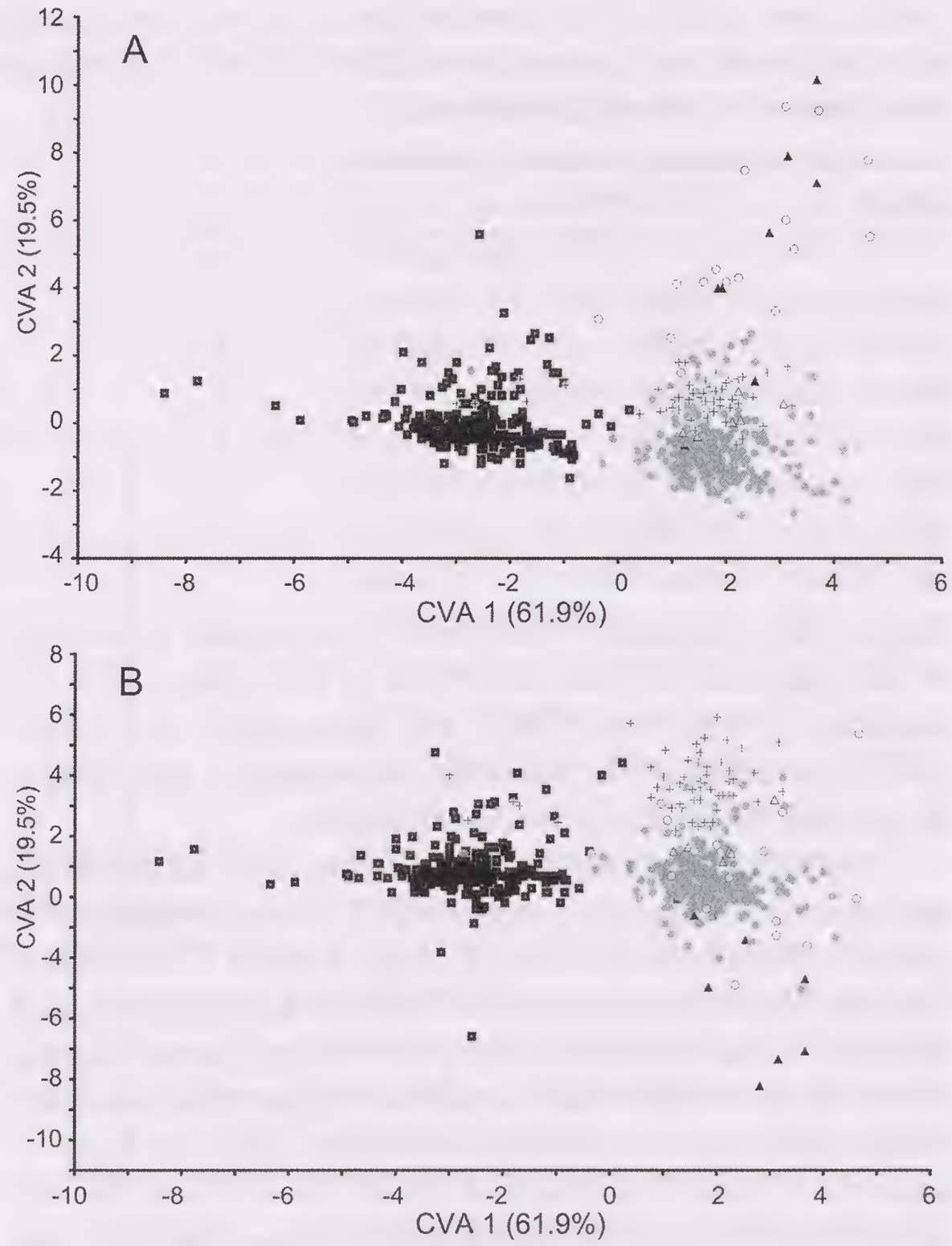

Figure 4. The distribution of the scored frequencies obtained from the CVA in the six groups. **A,** CVA 2 x CVA 1; **B,** CVA 3 x CVA 1. Symbols: ○ *Doryteuthis* (*Amerigo*) *opalescens*; ▲ *Lolliguncula* (*Lolliguncula*) *argus*; ■ *Lolliguncula* (*Lolliguncula*) *panamensis*; ● *Lolliguncula* (*Loliolopsis*) *diomedeae*; △ *Lolliguncula* sp. 1, and + *Lolliguncula* sp. 2.

genera, and subfamily) and problems at the species level, including cryptic species and the possibility of natural hybridization among neighbor species (Brakoniecki 1986, Vecchione *et al.* 2005, Cardoso and Hochberg 2013). In the current research, loliginid specimens from the western coast of Mexico were analyzed using morphologic and morphometric analyses. Organisms from the species *Pickfordiateuthis vossi* were not captured as this species inhabits very shallow areas near the coast (Jereb *et al.* 2010). However, this species can be easily identified by modified tentacles, which are similar in appearance to pairs of arms IV, although they do not show a tentacular club. The posterior fin lobes extend beyond the posterior end of the mantle and do not join posteriorly, unlike the remaining species from the region. Moreover, the buccal membrane does not have suckers, and the funnel organ has an inverted "U" shape (Brakoniecki 1996).

The funnel organ was a determining taxonomic characteristic for discrimination of the species and the two forms. This character has the advantage of not presenting differences regarding sex. Important differences regarding size, shape, and width of the ventral and dorsal patches were observed. In addition, these differences were easily distinguishable in organisms preserved in formaldehyde, but they were not visible in fresh organisms and organisms preserved in alcohol. In these cases, the use of methylene blue as an aid to emphasize the structures is recommended.

Our multivariate analysis supported the existence of four species, *Lolliguncula panamensis, Lolliguncula argus, Lolliguncula diomedeae,* and *Doryteuthis opalescens*, and two forms, *Lolliguncula* sp. 1 and *Lolliguncula* sp. 2. These last

Table 2. The classification matrix of species in the family Loliginidae. Abbreviations: Lp = *Lolliguncula* (*Lolliguncula*) *panamensis*; La = *Lolliguncula* (*Lolliguncula*) *argus*; Ld = *Lolliguncula* (*Loliolopsis*) *diomedeae*; Lsp1 = *Lolliguncula* sp. 1; Lsp2 = *Lolliguncula* sp. 2; and Do = *Doryteuthis (Amerigo) opalescens*.

Species	% Correct	No. of specimens classified						
		Do	La	Ld	Lp	Lsp1	Lsp2	Total
Do	75.00	12	2	1	1	0	0	16
La	75.00	0	6	2	0	0	0	8
Ld	94.39	0	0	219	1	1	11	232
Lp	97.19	0	1	1	208	1	3	214
Lsp1	33.33	0	0	4	0	2	0	6
Lsp2	90.74	0	0	0	5	0	49	54

shapes were assigned to the genus *Lolliguncula* according to the proposed criteria of Hoyle (1904), Berry (1911), Brakoniecki (1980 and 1986), and Brakoniecki and Roper (1985) and based on the following characteristics: body shape, fin size and shape, and a buccal membrane.

A discriminant analysis showed that using 15 variables, species could be classified accurately 77.6% on average: *Lolliguncula panamensis* (97.2%), *L. argus* (75%), *L. diomedeae* (94.4%), *Doryteuthis opalescens* (75%), and *Lolliguncula* sp. 2 (90.7%). In this regard, the high percentage of correct classification in the discriminant analysis suggests that the form *Lolliguncula* sp. 2 is morphometrically different from *L. panamensis* and *L. diomedeae*. These results and the observed differences in the funnel shape indicate that the form *Lolliguncula* sp. 2 could belong to a new undescribed species. In addition, the individuals grouped in the form *Lolliguncula* sp. 1 were weakly supported (33.3%) and based on the observed confusion in the classification matrix, the grouping was more similar to *L. diomedeae*. Nevertheless, the low percentage of classification of this species might have been affected by the small sample size. An increase in both sample size and number of sampling localities may bring better results. The proportions in the hectocotylus size of the form *Lolliguncula* sp. 1 were greater (115–125%) than that reported for *L. diomedeae* (76–107% ML), and for the form *Lolliguncula* sp. 2 (37–106%). Unfortunately, the hectocotylus was not evaluated in detail for the species and the two forms. Collateral analysis by reviewing the hectocotylus in a deeper detail, statolith geometric morphometrics, genetic analyses based on mitochondrial DNA, should be done on the specimens deposited in the Santa Barbara Museum of Natural History.

The observed confusion may result from the high intraspecific morphological variability, which increases with individuals of both sexes and different sizes. In this study, from the 15 analyzed variables, 11 were positive variables for species and two forms discrimination. This highly overlapping of characteristics among loliginid species has been widely

Table 3. The Factor Structure Matrix Correlation Variables of CVA of Loliginidae off the Pacific coast of Mexico. Variables with a larger contribution are emphasized in bold-faced font.

Measurement	CV1	CV2	CV3
Fin length (right)	0.171480	**0.385246**	**0.241742**
Fin length (left)	**0.465680**	**-0.235947**	-0.040737
Mantle length (dorsal)	**0.386146**	**-0.201109**	-0.067915
Middle mantle length	**-0.769860**	**0.263458**	-0.044251
Fin width	**0.246934**	0.092860	0.000141
Funnel length	0.133937	0.171190	0.080246
Funnell width	0.129915	**0.311112**	**-0.392916**
Head length	0.171110	0.137354	**0.277466**
Head width	**0.252350**	**0.490012**	**0.454467**
Eye diameter	0.159098	0.022863	-0.145151
Arm I length	0.734900	0.111758	0.183470
Arm II length	0.045018	0.088165	0.025650
Arm III length	0.123661	**0.381721**	0.037506
Tentacle length	0.111437	**0.794937**	**-0.224416**
Tentacular club length	**0.273415**	**0.547080**	**-0.215554**

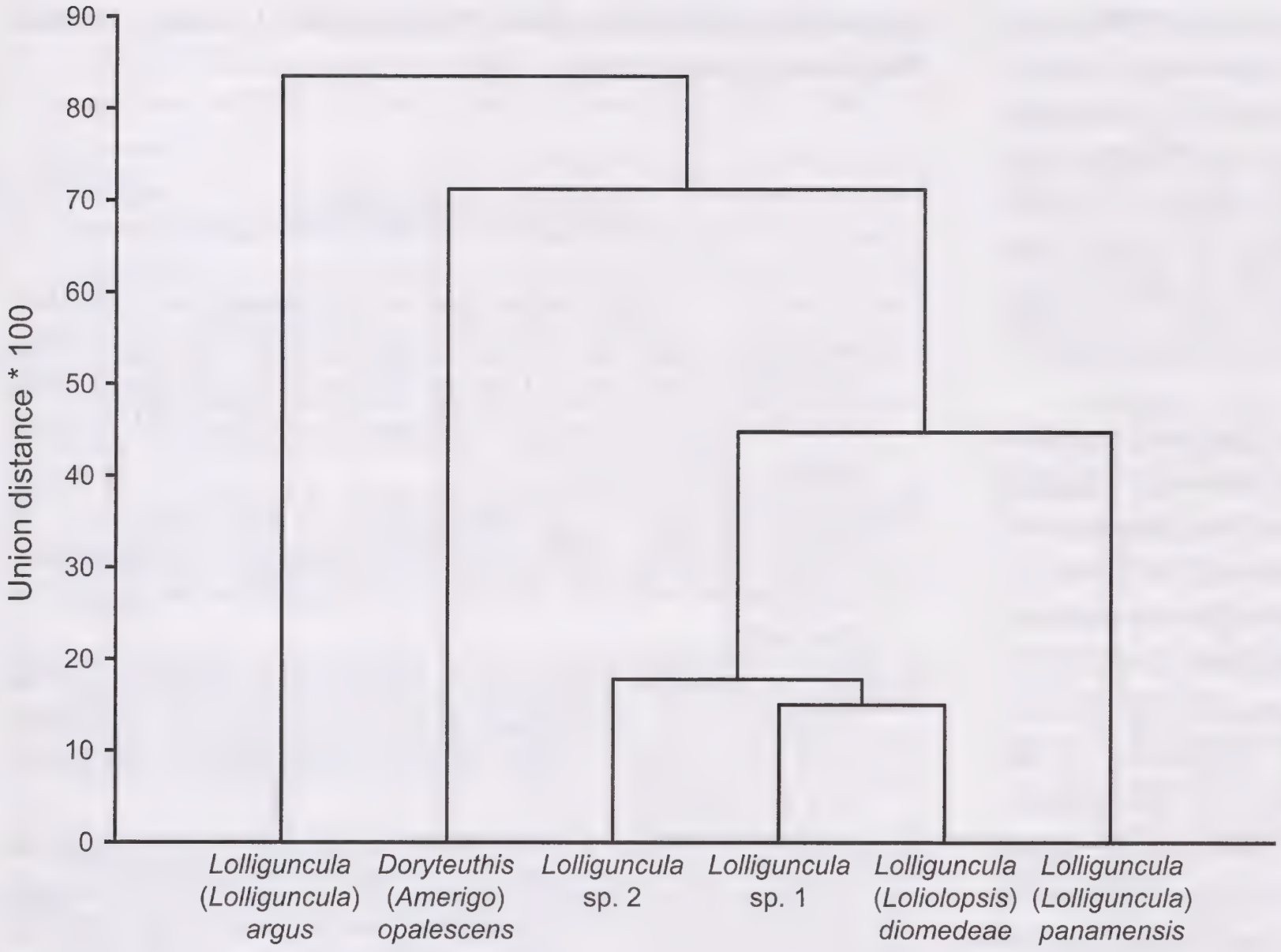

Figure 5. Dendogram based on the distances D2 of Mahalanobis and union UPGMA of *Doryteuthis (Amerigo) opalescens*, *Lolliguncula* (*Lolliguncula*) *argus*, *Lolliguncula* (*Lolliguncula*) *panamensis*, *Lolliguncula (Loliolopsis) diomedeae*, *Lolliguncula* sp. 1, and *Lolliguncula* sp. 2, off the Pacific coasts of Mexico and in the Gulf of California.

commented on by several authors (Anderson 2000a, 2000b, Vecchione *et al.* 2005).

Multivariate techniques have been shown to enhance species discrimination (Martinez *et al.* 2002). According to our analysis, high loading variables, particularly, *Lolliguncula panamensis*, differ from the others by longer middle mantle and fin lengths. Whereas *L diomedeae* and the form *Lolliguncula* sp. 2 differ in tentacle length, tentacular club length and head width from the remaining species. Lastly, *L. argus, D. opalescens*, and the form *Lolliguncula* sp. 1 differ from the other groups because of the following characteristics: head width, fin width, and head length. Based on the results of this research, although recent loliginid species identification guides (Roper *et al.* 1995, Sánchez 2003, Jereb *et al.* 2010) use the percentage of fin length to discriminate species, this criterion was not an important variable. Moreover, more than 50% of specimens showed one fin larger than the other; hence measurements of both fins were obtained from organisms in the present study.

Additionally, the presence of sexual dimorphism in the group makes identification more complex, thus requiring separate descriptions for males and females. In males the hectocotylus has a great taxonomic value to separate species (Brakoniecki 1986); however, in females there is no analogous organ with such taxonomic value. *Lolliguncula argus* males share a cylindrical mantle shape with small and narrow fins with *L. diomedeae*, but differ from *L. diomedeae* and the remaining species by presenting the hectocotylus in the left ventral arm. *Lolliguncula diomedeae* and *L. panamensis* males differ from one another in the arms. *Lolliguncula diomedeae* has two modified ventral arms: the left arm is proportionally larger (76-107% ML) than that of *L. panamensis* (42-69% ML). Moreover, the length relationships of mantle-mantle width from these species show obvious differences: *L. diomedeae* is "thinner" than *L. panamensis*; in other words, its body has a more fusiform shape (Sánchez 2003). Lastly, in *Doryteuthis opalescens*, the mantle is cylindrical with large and triangular fins.

Hoyle's 1904 description of *Loliolopsis diomedeae* was based on a female captured in Acapulco, Guerrero, Mexico, 1891. This description showed that more information was necessary for the accurate and complete identification of the species, according to the species description criteria of Roper and Voss (1983). In Hoyle's 1904 original description, the specimen's mantle length was not considered. This measurement is perhaps the most important in a description because comparisons can be performed with other organisms and proportions of the different body structures can be obtained from the specimens. This absence of basic data was also evident in the description of *Lolliguncula panamensis* (Berry 1911). Berry only analyzed three females (85, 101 and 105 mm ML) when describing the species, and the data presented only referenced the location where the organisms were captured (Panama) and the location of the type specimens (Stanford University Museum).

Further Brakoniecki (1980) described *Lolliguncula tydeus* as a new species based on specimens in the collections of the Rosenstiel School of Marine and Atmospheric Science of the University of Miami, which were catalogued as *Loliolopsis diomedeae* (Hoyle, 1904). This author analyzed males and females of different sizes (26-53 ML), determined a detailed morphological description of the internal and external structures, and separated the species based mainly on differences in the hectocotylus shape. However, after examining larger collections in 1986, Brakoniecki concluded that *L. tydeus* was the male of *L. panamensis* and was therefore a junior synonym of *L. panamensis*.

For the tropical eastern Pacific, Roper *et al.* (1994) presented an illustrated identification guide for four species: *Doryteuthis* (*Amerigo*) *opalescens*, *Lolliguncula* (*Loliolopsis*) *diomedeae*, *Lolliguncula* (*Lolliguncula*) *argus*, and *Lolliguncula* (*Lolliguncula*) *panamensis*. Nonetheless, the authors did not provide a basis for a precise discrimination of genera and subgenera. Moreover, the diagnostic characteristics were based on adult specimens; therefore, young specimen identification remained confusing.

Based on the results of the present and previous research on the group, we recognize that there are at least five loliginid species in the Mexican Pacific, *Lolliguncula* (*Lolliguncula*) *panamensis*, *Lolliguncula* (*Lolliguncula*) *argus*, *Lolliguncula* (*Loliolopsis*) *diomedeae*, *Doryteuthis* (*Amerigo*) *opalescens*, and *Pickfordiateuthis vossi* (Roper *et al.* 1995; Jereb *et al.* 2010). From the four identified species in this research, *Lolliguncula panamensis* and *L. diomedeae* are widely distributed in the Eastern Tropical Pacific. The distribution of *Doryteuthis opalescens*, with temperate affinity, is reported from the south western coast of Baja California Peninsula, Mexico to southeastern Alaska, U.S.A. *Lolliguncula argus* is restricted to the northwest Mexican Pacific. Recently there was a report on the presence of a population on the west coast of Baja California Sur and in the Gulf of California, approximately 950 km off its known distribution range (Granados-Amores *et al.* 2013). To validate the species treated herein as *Lolliguncula* sp. 1 and *Lolliguncula* sp. 2 a detailed morphological description, including the hectocotylus description is needed. In addition, field evaluations are required to precisely determine their distribution ranges.

According to the analysis developed in the present research, there were morphologic and morphometric characteristics that indicated a greater diversity of species compared to what was previously reported for the Mexican Pacific. However, it is necessary to increase the sample size for some species and explore innovative and powerful tools such as geometric morphometry in statoliths, which has provided good results for separating teuthid, octopod and cuttlefish species, including some loliginid species of the Mediterranean Sea (Dommergues *et al.* 2000, Lombarte *et al.* 2006, Neige 2006). Genetic and molecular analyses can also be useful for cryptic species identification (Vecchione *et al.* 2005).

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